

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: )  
Libutti *et al.* ) Art Unit: 1632  
Application No. 10/510,652 ) Examiner: Singh, Anoop Kumar  
Filing Date: October 28, 2004 ) Confirmation No. 4377  
For: QUANTITATIVE ASSAY OF THE )  
ANGIOGENIC AND ANTIANGIOGENIC )  
ACTIVITY OF A TEST MOLECULE )

DECLARATION OF FRANK CUTTITTA UNDER 37 C.F.R. § 1.132

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

NEEDLE & ROSENBERG, P.C.  
Customer Number 36339

Sir:

I, Frank Cuttitta, hereby declare that:

1. I am a permanent intramural scientist at National Institutes of Health, and hold a Ph.D. in Immunology/Biochemistry from the University of Maryland. I have over thirty-seven years experience in the biological sciences with the U.S. Federal Government, with an emphasis on immunology and peptide chemistry, and over twenty-seven years experience in the study of peptide growth factors. This includes specific experience with biomediators that drive neovascularization events in the normal and disease setting. I am the Director of the Angiogenesis Core Facility at the National Cancer Institute. A partial curriculum vitae is attached to this declaration.

2. I have reviewed the specification of the above-identified application. Briefly, Dr. Libutti has provided a modified CAM assay for measuring the angiogenic or antiangiogenic activity of a sample. This method involves directly injecting the sample and a fluorescent-labeled

particle into a vessel of the CAM prior to removing the region of interest from the egg. A three-dimensional image of this region is then captured and the resulting pixels quantified to obtain a fluorescent vascular density (FVD) value that can be compared for control and treated CAMs.

3. I have further read and understood Brooks et al. (Science. 1994. 264:570-571), Roberts et al. (Cancer Res. 1992. 52(4):924-30), Kimel et al. (SPIE. 1996. 2628:69-76) and Rizzo et al. (Microvascular Res. 1995. 49-63).

4. It is my opinion that the Libutti quantitative CAM technology disclosed and claimed in the above referenced application provides substantially superior results to that of Brooks et al. or any other previously available CAM assay. For example, as presented in Miller et al. (J. Trans. Med. 2004. 2(1):4, attached hereto) and as also shown in the above referenced application in Comparative Example 1, paragraphs [0069] to [0077], the Libutti et al. CAM assay was capable of distinguishing a significant increase in vascular density caused by bFGF (1  $\mu\text{g/ml}$ ) over the control, whereas the CAM assay taught by Brooks et al. showed no difference between treated and control tissue. This contradicted the prevailing wisdom in this field by identifying for the first time the scope of the inaccuracy of the gold standard of the time, the Brooks et al. CAM assay. These improved results are in part due to the fact that the Libutti et al. method is an objective base quantification of vascular density and not a subjective interpretations of dye "retention" (Robert et al. and Rizzo et al.), dye "biodistribution" (Kimel et al.) or dye "density" (Brooks et al.). None of the cited methods involved objective quantification. Most importantly, the Libutti precedent of introducing anti-angiogenic reagents intravenously in the CAM as a mimetic to clinical studies with patients is a ground-breaking approach well recognized by other scientists/clinicians in the field. Moreover, one of the limitations of the Brooks et al. method is its slowness, a result of using blinded human readers. This, made it

unsuited to assaying large numbers of compounds for potential anti-angiogenic or angiogenic activity. In contrast the Libutti et al. method is capable of high throughput because of its accuracy and automation.

5. I further declare that prior to the filing of the above referenced application, those working in the angiogenesis field did not have reason to believe that the CAM assay taught by Brooks et al. was unable to accurately quantitate angiogenesis (increase in vascular density) or that objective quantitation would provide significantly superior results. Prior to Libutti et al. method (claimed in the present application), CAM assays as taught by Brooks et al. were routinely being used to quantitate the effect of agents on angiogenesis. While this assay was understood to have limitations, there was no general recognition in the field that results based on dye density were an inaccurate indicator of vessel density, or that a different approach would give significantly more accurate results.

7. I further declare that these superior results were surprising to scientists in this field when they were first disclosed after the above application was filed. While some improvement of accuracy was expected with the use of more quantitative measurements of angiogenesis, the significance of the improvement shown by the Libutti et al. method was unexpected. This is evidenced by the fact that prior to the publication of the Libutti et al. assay, no one had successfully attempted to use such complex methods even though direct injection of fluorescent molecules was already being used in other contexts. This is because the predicted benefits of objective quantification were not anticipated to outweigh the technological hurdles of using angiography on a CAM assay.

8. I further declare that Libutti et al. overcame significant technological hurdles to achieve these superior results. These hurdles include determining which vein was best used as

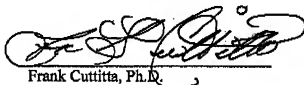
the injection site, identifying what size needle to used when introducing anti-angiogenic drugs to minimize leakage (backwash of drug into extracellular space) and thus maintain consistency in delivery dosage, determining drug toxicity level of the CAM, identifying what is the best size FITC-dextrin (of the multiple sizes offered by Sigma) to maximize retention time of dye, work out what was the best time to harvest the CAM disk harvest after FITC intravenous injection to maximize fluorescent signal, what was the best fixative to use, what was the appropriate storage temperate to retain maximal fluorescent intensity of vascular network, identify what was the most robust system to use for quantitating fluorescence intensity (pixel expression) of vessels and finally, doing repetitive runs of multiple drugs (fumagillin, LM609, CC5079) to validate assay reliability/efficiency. However, once these challenges were overcome and the assay validated by Libutti et al., no more than routine effort was needed by a scientist working in the angiogenesis field to modify each of the above aspects of the method.

9. I further declare that the Libutti et al. assay has been commercially successful based on the recognition of the superior results obtained using the objective and direct quantitation of vessel density. The results obtained by Libutti et al. with the claimed method have been lauded by others in the field in the face of competing methods. I and others are now routinely using the claimed Libutti et al. CAM assay. In fact, Libutti's CAM assay is now taught as a major session in the NCI Angiogenesis Core Facility sponsored NIH/Foundation for Advanced Education in the Sciences (FAES) four-day wet-lab course offered twice a year at the Cloister Facility on the main NIH campus in Bethesda, MD for intramural/extramural attendees. Moreover, this commercial success is directly related to the superior results of this assay over the methods previously used.

10. Until the development of the claimed assay, nobody had been able to successfully measure angiogenesis to a level of speed and accuracy that allowed thoughtful consideration of possible modulators of angiogenesis. Furthermore, the degree of accuracy achieved by Libutti et al. was unprecedented. Finally, the Libutti CAM approach of introducing anti-angiogenic drugs intravenously to ascertain their efficacy for suppressing mediator-induced neovascularization was a unprecedented undertaking and a critical mimetic model to clinical trials on cancer patients. Therefore, it is my belief that the claimed methods significantly advanced the field of high throughput angiogenesis assays.

I declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be true, and further, that the statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 12/23/07



Frank Cuttitta, Ph.D.